

Preparation of Different Buffer Solutions

Buffers:

- All biochemical reactions occur under strict conditions of the concentration of hydrogen ion.
- Biological life cannot withstand large changes in hydrogen ion concentrations which we **measure as the pH**.
- Those solutions that have the ability to resist changes in pH upon the addition of **limited amounts** of acid or base are called **BUFFERS**.

Two types of Buffers

A buffer is made up of a **weak acid** and its conjugate base.

Or

A **weak base** and its conjugate acid.

Acidic Buffer

Are made from weak acid and its conjugated base [its salt].

Example:

1. $\text{CH}_3\text{COOH} / \text{CH}_3\text{COONa}$ (Pka)

→ CH_3COOH (Weak acid)

→ CH_3COONa (conjugated base –its salt-)

2. $\text{NaH}_2\text{PO}_4 / \text{Na}_2\text{HPO}_4$ (Pka)

Basic Buffer

Are made from weak base and its conjugated acid [its salt].

Example:

1. $\text{NH}_3 / \text{NH}_4\text{Cl}$ (Pkb)

→ NH_3 (Weak base)

→ NH_4Cl (conjugated acid –its salt-)

Mechanism of Action:

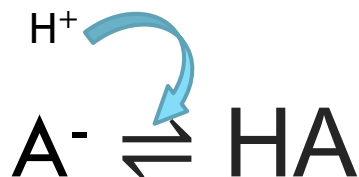
□ How buffers can resist the change in pH?

-Example using [HA/A⁻] buffer:

→ Where: HA is Weak acid and A⁻ is conjugated base [its salt].



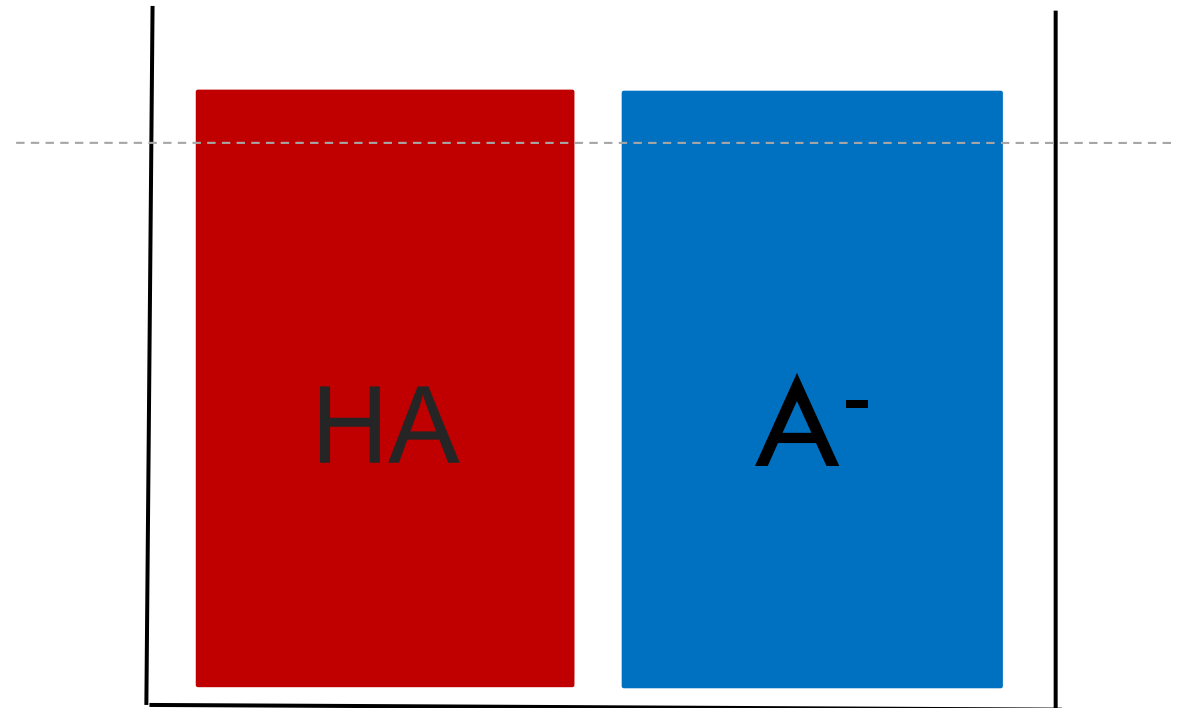
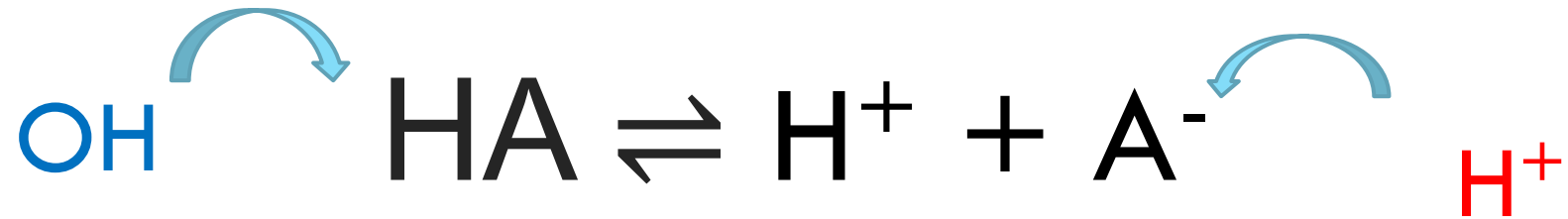
If H⁺ (acid) is added to this buffer system → H⁺ will react with conjugated base → to give conjugate acid.



If OH⁻ (base) is added to this buffer system → OH⁻ will react with conjugated acid → to give conjugate base and H₂O.



Mechanism of Action



Mechanism of Action cont':

□ Example:

□ Buffer system: CH_3COOH / CH_3COO^-

conjugated base

CH_3COO^-

conjugated acid

CH_3COOH

□ When acid $[\text{H}^+]$ added:



□ When base $[\text{OH}^-]$ added:



□ NOTE: It resists pH changes when its two components are present in **specific proportions**.

→ Thus the buffer is effective as long as it does not run out of one of its components. (There are enough conjugated base and conjugated acid to absorb the H^+ ions or OH^- ions added to the system respectively).

Henderson-Hasselbalch equation:

- It is often used to perform:
 1. To calculate the pH of the Buffer.
 2. To preparation of Buffer.

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

- It relates the K_a [dissociation constant] of a weak acid , $[HA]$ concentration of weak acid component , $[A^-]$ concentration of conjugate base [salt of the weak acid] component and the pH of the buffer.
- The equation is derived from the acid dissociation constant.

Henderson-Hasselbalch equation cont':

- A buffer is **best used close to its pKa** [to act as a good buffer the pH of the solution must be within one pH unit of the pKa].

→ The buffer capacity is optimal when the ratio of the weak acid to its salt is 1:1; that is, when pH = pKa

$$\text{pH} = \text{pka} + \log 1$$

$$\text{pH} = \text{pka} + 0$$

$$\text{pH} = \text{pka}$$

Calculating the pH:

For weak acid [not buffers]

$$\text{pH} = \frac{\text{pk}_a + \text{p}[\text{HA}]}{2}$$

* $\text{P}[\text{HA}] = -\log [\text{HA}]$

For weak base [not buffers]

$$\text{pOH} = \frac{\text{pk}_b + \text{p}[\text{OH}]}{2}$$

* $\text{P}[\text{OH}] = -\log [\text{OH}]$

For buffers

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

$$\text{pH} = \text{pkw} - \text{pOH}$$

[Pkw : number of dissociation constant of H₂O].

Practical Part

Objectives:

- To understand the behaviour and nature of buffers solutions.
- To learn how to prepare buffers.

A) Nature of buffers:

Method:

1. You are provided with: 0.2M solution of CH_3COOH , 0.2M solution of CH_3COONa .

→ Determine which is the weak acid and which is the conjugated base [or its salt].

2. Calculate the volume that you must take from CH_3COOH and CH_3COONa to prepare the following mixtures with final **volume of the solution =20 ml** :

1. 100% [HA]

2. 75% [HA] , 25% [A^-]

3. 50% [HA] , 50% [A^-]

4. 25% [HA] , 75% [A^-]

3. Calculate the pH for each solution [pK_a of $\text{CH}_3\text{COOH} = 4.76$].

4. Follow the table.

Calculations:

1. To Calculate the volume that you must take from CH_3COOH and CH_3COONa to prepare the previous mixtures with final volume of the solution =20 ml:

(A) 100% [HA]:

The final volume is 20ml, So:

$$20 \times 100\% = (20 \times 100)/100 = 20 \text{ ml}$$

→ Take 20ml of HA and measure the pH.

(B) 75% [HA], 25% [A⁻]:

$$\text{From HA} = 20 \times 75\% = (75 \times 20) / 100 = 15 \text{ ml}$$

$$\text{From A}^- = 20 \times 25\% = (25 \times 20) / 100 = 5 \text{ ml}$$

→ Mix 15ml HA and 5 ml A⁻ and measure the pH (measured PH) note that the total volume is 20 ml [15ml +5ml =20ml]

□ The same way for other mixtures ...

Note:

HA : as CH_3COOH .

A⁻ : as CH_3COONa .

Calculations cont':

2. To Calculate the pH for the previous mixtures with pKa of CH₃COOH = 4.76 :

(A) 100% [HA]:

$$\text{pH} = \frac{(\text{pKa} + \text{p[HA]})}{2} \quad \rightarrow \quad \text{p[HA]} = -\log 0.2 = 0.69 \quad \rightarrow \quad \text{pH} = \frac{(4.76 + 0.69)}{2} = \underline{2.72}$$

(B) 75% [HA], 25% [A⁻]:

$$\text{pH} = \text{pka} + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad \rightarrow \quad \text{pH} = 4.76 + \log \frac{[\text{A}^-]}{[\text{HA}]} \quad \rightarrow \quad [\text{HA}] = C_1 \times V_1 = C_2 \times V_2$$
$$= 0.2 \times 15 = C_2 \times 20 = \underline{C_2 = 0.15\text{M}}$$

$$\rightarrow [\text{A}^-] = C_1 \times V_1 = C_2 \times V_2$$
$$= 0.2 \times 5 = C_2 \times 20 = \underline{C_2 = 0.05\text{M}}$$

So, $\text{pH} = 4.76 + \log 0.05/0.15 \rightarrow \text{pH} = \underline{4.282}$

Calculations cont':

(C) 50%[HA], 50%[A⁻] :

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \rightarrow \text{pH} = 4.76 + \log \frac{[\text{A}^-]}{[\text{HA}]} \rightarrow [\text{HA}] = C_1 \times V_1 = C_2 \times V_2 \\ = 0.2 \times 10 = C_2 \times 20 = C_2 = 0.1\text{M}$$

$$\rightarrow [\text{A}^-] = C_1 \times V_1 = C_2 \times V_2 \\ = 0.2 \times 10 = C_2 \times 20 = C_2 = 0.1\text{ M}$$

So, $\text{pH} = 4.76 + \log 0.1/0.1 \rightarrow \text{pH} = 4.76 + 0 = \mathbf{4.76}$ [pH=pka]

(D) 25% [HA], 75% [A⁻] :

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]} \rightarrow \text{pH} = 4.76 + \log \frac{[\text{A}^-]}{[\text{HA}]} \rightarrow [\text{HA}] = C_1 \times V_1 = C_2 \times V_2 \\ = 0.2 \times 5 = C_2 \times 20 = C_2 = 0.05\text{M}$$

$$\rightarrow [\text{A}^-] = C_1 \times V_1 = C_2 \times V_2 \\ = 0.2 \times 15 = C_2 \times 20 = C_2 = 0.15\text{ M}$$

So, $\text{pH} = 4.76 + \log 0.15/0.05 \rightarrow \text{pH} = \mathbf{5.24}$

Result:

Solutions	HA CH ₃ COOH (ml)	A ⁻ CH ₃ COONa (ml)	Final volume (ml)	Calculated pH	Measured pH	2M HCl (ml)	Measured pH	The difference
100%[HA]			20 ml			0.1		
75%[HA],25%[A ⁻]			20 ml			0.1		
50%[HA],50%[A ⁻]			20 ml			0.1		
25%[HA],75%[A ⁻]			20 ml			0.1		

B) Preparation of buffer:

- You are provided with **0.2M acetic acid** and **solid sodium acetate**. Prepare 50ml of a 0.19M acetate buffer pH =4.86 if you know that (pKa=4.76).

Calculations:

- Solid sodium acetate [as A⁻].
- 0.2M Acetic acid [as HA].
- Pka = 4.76

Provided

- Final volume of buffer =50ml
- pH=4.86
- Buffer concentration = 0.19 M
- Buffer Concentration = [HA] + [A⁻]
 $0.19 = [HA] + [A^-]$

Required

Calculations cont':

- To prepare a buffer Henderson-Hasselbalch equation is used:

$$\text{pH} = \text{pka} + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

- First calculate the concentration of the weak acid and its conjugated base that make up the buffer with 0.19M:

→ Assume $[\text{A}^-] = y$ and $[\text{HA}] = 0.19 - y$

So:

$$4.86 = 4.76 + \log \frac{y}{0.19 - y}$$

$$0.1 = \log \frac{y}{0.19 - y} \quad \rightarrow \text{by taking the "Anti log for both sides"} \quad \rightarrow \quad 1.26 = \frac{y}{0.19 - y}$$

$$y = 1.26 \times (0.19 - y) \quad \rightarrow \quad y = 0.24 - 1.26y \quad \rightarrow \quad y + 1.26y = 0.24 \quad \rightarrow \quad 2.26y = 0.24$$

$y = 0.11 \text{ M}$ [which is the concentration of $[\text{A}^-]$ in the buffer]

So,

$$\begin{aligned} [\text{HA}] &= 0.19 - 0.11 \\ &= \underline{0.08 \text{ M}} \quad \text{[which is the concentration of } [\text{HA}] \text{ in the buffer]} \end{aligned}$$


$$0.11 + 0.08 = 0.19 \text{ M}$$

Calculations cont':

- To calculate the volume needed from [HA] to prepare the buffer, No. of mole of [HA] should be calculated first:

No. of mole = Molarity x Volume of solution in L

$$= 0.08 \times 0.05 = 0.004 \text{ mole}$$

So, M of stock = no. of mole / Volume in Liter

$$0.2 = 0.004 / V$$

→ $V = 0.02 \text{ L} = \underline{20 \text{ ml}}$

- To calculate the weight needed from [A-] to prepare the buffer, No. of mole of [A-] should be calculated first:

No. of mole = Molarity x volume of solution in L

$$= 0.11 \times 0.05 = 0.0055 \text{ mole}$$

weight in (g) of [A-] = No. of moles x MW

→ $= 0.0055 \times 82 = \underline{0.451 \text{ g}}$

Method:

- Now take 20 ml from 0.2M acetic acid and 0.451 g from solid sodium acetate and then complete the volume up to 50 ml by addition of water.

C) Testing for buffering behaviour:

- In one beaker add 10ml of 0.19M acetate buffer that you have prepared, and in another beaker add 10ml of 0.2M KCl.
- Measure the pH.
- Add 0.1ml from 2M HCl to for both solutions.
- Measure the pH after the addition.

Solution	Measured pH	Add 2M HCl	Measured pH
0.19M acetate buffer		0.1 ml	
0.2M KCl		0.1 ml	